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Understanding the genetic basis of cancer will greatly improve our ability to develop more effective therapies. One family of genes involved in cancer is the tumor suppressor genes. We have functional evidence for a new breast cancer suppressor gene on chromosome 17. Transfer of a normal human chromosome 17 results in growth arrest of the MCF7 breast cancer cell line. We are using two approaches to localize the region containing this gene. The first involves the transfer of chromosome 17s containing specific deletions into MCF7 cells followed by our growth arrest assay to determine whether that chromosome contains the suppressor gene. We have determined that the gene lies on chromosome 17q and are now further localizing this gene. In parallel, we are studying loss of heterozygosity (or LOH, which is an indirect indicator of the presence of a suppressor gene) in breast tumors and have identified at least three candidate LOH regions on chromosome 17. Future studies will involve physical mapping of the region containing the suppressor gene, and we provide evidence for our ability to develop region-specific markers. We have also undertaken feasibility studies to determine whether YACs can be used in the MCF7 growth arrest assay.

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Graham Casey 8/4/95

PI - Signature Date

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INTRODUCTION:

Understanding the genetic mechanisms of cancer development will greatly improve our chances of developing more effective therapies. Cancer arises in part from mutations in critical genes in a single cell, and one class of genes involved are the tumor/growth suppressor genes. This is a functional definition for a gene whose product can suppress the tumorigenic behavior/growth of the cell in which it is expressed. The presence of suppressor genes can been inferred functionally by cellular phenotype changes in somatic cell fusion experiments, and by the introduction of single human chromosomes by microcell-mediated chromosome transfer (MMCT). The presence of a gene(s) involved in growth suppression is implicated when specific changes in cell growth are observed after that chromosome is introduced into a cancer cell line.

We have functional evidence for a breast cancer growth suppressor gene on chromosome 17. Growth of the MCF7 breast cancer cell line is arrested in vitro following the transfer of a normal human chromosome 17. The p53 tumor suppressor gene is not responsible for this growth arrest, as we have previously shown that MCF7 cells contain a structurally wild-type p53 gene, and are unaffected by the overexpression of exogenous wild-type p53 cDNA. Our hypothesis is that we have functionally identified a new growth suppressor gene, and that this gene may be inactivated, leading to uncontrolled growth in some breast cancers.

Our approach is to combine functional and physical approaches to identify the gene on chromosome 17 responsible for in vitro growth arrest. We propose to functionally localize the MCF7 growth suppressor gene region by introducing chromosome 17s containing specific deletions into MCF7 cells by microcell transfer. This approach will be complemented by loss of heterozygosity analysis of primary breast tumors using polymorphic markers that map to the same region. Following localization, positional cloning approaches will be used to map this region, followed by methods to identify expressed sequences. Candidate genes will be functionally assessed by DNA transfection, and the significance of this gene in the development of breast cancer will be assessed. The experiments we propose maximize the potential for mapping and cloning the MCF7 growth suppressor gene(s) by combining our ability to assess functional characteristics of specific candidate genes and standard positional cloning techniques.

BODY:

Progress in four specific areas of research is summarized below:

1. Functional localization of the MCF7 growth suppressor gene. We have been developing a panel of hybrids that contain deletions in regions of chromosome 17 that encompass genes or putative genes implicated (by LOH studies) in breast cancer development. These regions/genes include 17p13.3, p53, BRCA1 (and the surrounding region), and two regions of LOH on 17q21-qter. These hybrids have been developed by the transfer of a neomycin-tagged chromosome 17 into the mouse cell line A9 by microcell transfer, where a low proportion (approximately 5%) of resulting hybrids contain deletions in chromosome 17. Such deletions occur at random, and are characterized initially using a panel of chromosome 17 microsatellite and STS markers. The presence of a single intact chromosome 17 is confirmed by FISH (fluorescence in-situ hybridization) chromosome analysis.

Our functional assay predicts that a chromosome containing the MCF7 growth suppressor gene will result in in vitro growth suppression of MCF7 cells, whereas a chromosome containing a deletion encompassing this gene will have no effect upon the growth of MCF7 cells and will be retained in MCF7/chromosome 17 hybrids.

Our initial strategy has been to localize the MCF7 growth suppressor gene to the short (17p) or long (17q) arm of chromosome 17 using deletion chromosome 17 hybrids. Hybrid 43 contains a deletion encompassing the 17p13.3 LOH region and p53, and results in in vitro growth suppression of MCF7 cells following microcell transfer. Figure 1 shows an example of MCF7 hybrids analyzed for the presence of the introduced chromosome following transfer of hybrid 43 using marker SSTR2 (located at 17q23). These data confirm our previous p53 transfection studies excluding p53 as the gene responsible for the MCF7 growth suppression. Deletion hybrid 26 contains 17q only and also resulted in growth suppression, confirming localization of the growth suppressor gene to 17q. We are now using a series of hybrids to further localize the gene on 17q and are continuing to develop other hybrids for further refinement of its location. For example, we are screening for a hybrid that encompasses the BRCA1 gene. Microcell transfer of this chromosome will indicate whether BRCA1 or a second tumor suppressor gene in the region (which is suggested by the high LOH in the BRCA1 region but absence of BRCA1 mutations, in sporadic breast tumors) is involved in growth suppression. We are also sequencing BRCA1 in MCF7 cells. If a mutation is identified we will perform DNA transfection studies of BRCA1 cDNA. In addition to this hybrid, we have developed 5 hybrids that contain deletions dividing 17q21-q24 into approximately 12 regions. These hybrids will enable the functional localization of the growth suppressor gene to a region less than 5cM

which is our target before we begin regional mapping. Figure 2 shows an ideogram of a series of monochromosome 17 hybrids that we have fully characterized using a panel of over 40 microsatellite markers and by FISH analysis and are in the process of transferring into MCF7 cells.

Following functional localization of the region containing this gene, we will develop YAC and cosmid contigs of the region, followed by isolation of expressed sequences. We are currently developing these positional cloning strategies focusing on chromosome 17p13.3. We have previously shown that this region contains a putative breast cancer tumor suppressor gene by loss of heterozygosity studies (Stack et al, 95). We have developed a YAC contig of this region and are currently using IRS-bubble PCR to generate probes to isolate cosmids from a chromosome 17-only cosmid library (supplied by Dr. Larry Deavon, Los Alamos) in order to develop a cosmid contig. The results of these studies are incomplete and will be provided in the next years report.

2. Chromosome 17 loss of heterozygosity studies. To complement our functional studies we are performing LOH studies to physically localize the gene. We have collected over 150 breast tumor/normal tissue pairs, and are performing LOH analyses of regions 17p13.3 and 17q21-qter. We have identified at least three regions of LOH on chromosome 17q in agreement with published data, and we are currently establishing the smallest regions of loss. Figure 3 shows an example of our loss of heterozygosity studies using marker CHRNB1. The results of the LOH studies are incomplete and upon completion will be provided in the next years report.

3. Positional cloning of the chromosome 17 region containing the MCF7 growth suppressor gene. Following localization of the MCF7 growth suppressor gene, we will begin physical mapping of the region. In addition to identifying known markers from the region we will develop additional region-specific markers. To provide evidence that we can generate region-specific markers, we have used a radiation-reduced hybrid containing only 17p13. Resulting IRS-PCR fragments were cloned by either TA or CUA cloning. Primers were designed from one clone which contained CA repeats, and we subsequently showed that this marker (D17S1174) mapped to 17p13.3 using our monochromosome hybrid panel. We have shown that this microsatellite has 7 alleles with a heterozygosity of 82%. This marker is being used in our LOH studies of the 17p13.3 region (Stack et al, 1995). Information on this marker is freely available through Genome DataBase.

4. The use of YACs in functional studies. We have been studying the use of YACs for future functional analyses of the MCF7 growth suppressor gene. Once a YAC contig of the region containing the MCF7 growth suppressor gene has been obtained, we propose the transfer of individual YACs for complementation studies. If the YAC contains the MCF7

growth suppressor gene, transfer should result in growth arrest of MCF7 cells. To test the feasibility of this approach we must initially achieve two objectives: a) show our ability to retrofit or introduce a selectable marker into YACs to enable selection following cell transfer; b) show our ability to stably transfer YACs into MCF7 cells. To achieve the first objective, we have obtained several plasmids from Zeneca which enable the retrofitting of YACs (Riley et al, 1992). Using these plasmids, we have shown that we can successfully retrofit YACs, and introduce the neomycin gene in either YAC arm. Experiments to show that YACs can be stably introduced into MCF7 cells are currently in progress.

CONCLUSIONS:

There are currently no modifications to our original plan for the identification of the MCF7 growth suppressor gene. In our original proposal we projected that we would have functionally localized the region containing this gene by the end of year 2. Based upon our current progress, we feel that this remains a viable projection. Once we have mapped the gene to a region of less than 5cM we will identify region-specific YACs, cosmids and phage using available region-specific markers or markers that we develop from radiation-reduced hybrids. Throughout the physical mapping phase we will continue to develop deletion hybrids and perform LOH studies to functionally and physically reduce this region further. Depending upon the size of the deletion encompassing the gene, and therefore the corresponding number of YACs, we will begin isolation of expressed sequences by exon trapping, cDNA library screening and solution hybrid capture methods. We will also pursue any candidate genes that are mapped to this region by DNA transfection studies.

REFERENCES:

Stack, M., Jones, D., White, G., Liscia, D.S., Venesio, T., Casey, G., Crichton, D., Varley, J., Mitchell, E., Heighway, J., and Santibanez-Koref, M. Detailed loss of heterozygosity analysis reveals that a tumour suppressor gene involved in sporadic breast cancer maps to the distal region of chromosome band 17p13.3. Human Molecular Genetics. In Press, 1995.

Riley, J.H., Morten, J.E.N., and Anand, R. Targeted integration of neomycin into yeast artificial chromosomes (YACs) for transfection into mammalian cells. Nucl. Acid Res., 20, 2971-2976, 1992.

APPENDIX:

1. Bibliography:

Abstracts:

None.

Manuscripts:

1. Stack, M., Jones, D., White, G., Liscia, D.S., Venesio, T., Casey, G., Crichton, D., Varley, J., Mitchell, E., Heighway, J., and Santibanez-Koref, M. Detailed loss of heterozygosity analysis reveals that a tumour suppressor gene involved in sporadic breast cancer maps to the distal region of chromosome band 17p13.3. Human Molecular Genetics. In Press, 1995.

2. Personnel:

Mark Paris, technician. This is a change from the original budget. Dr. Santibanez-Koref left the laboratory to return to Germany prior to funding commencing. Mark Paris was his replacement.

3. Budget Note:

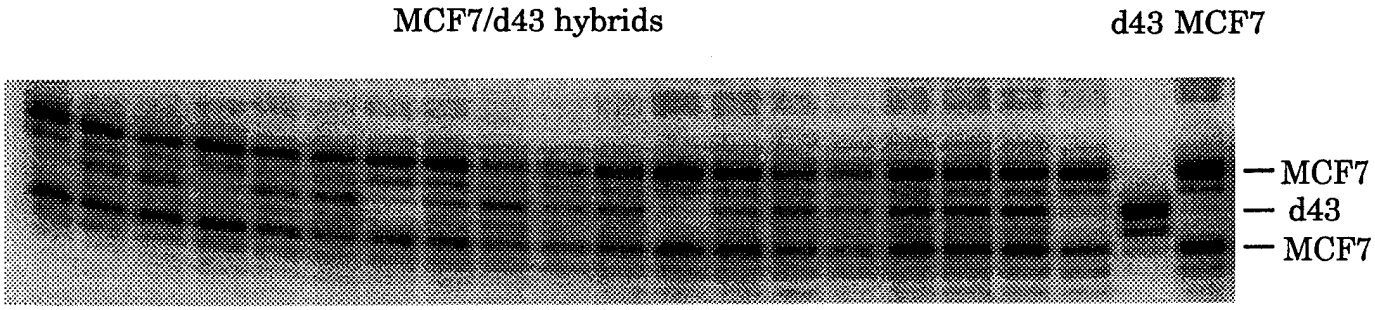
This project is funded jointly by NIH grant RO-1 CA61873 and the present award. Both agencies have been made aware of this from the beginning. The DOD budget covers those parts of the budget that the NIH did not fund. There is no duplication of funding. The report presented covers work conducted on the entire project. The DOD grant covers four years of funding, but for the first three years only covers a half-time person.

4. Figures:

1. Microsatellite analysis of MCF7/deletion 43 hybrids
2. Microcell hybrids currently available for microcell transfer into MCF7 cells
3. Loss of heterozygosity analysis of breast tumor/normal tissue pairs using marker CHRNB1

FIGURE 1

Microsatellite analysis of MCF 7/deletion 43 hybrids



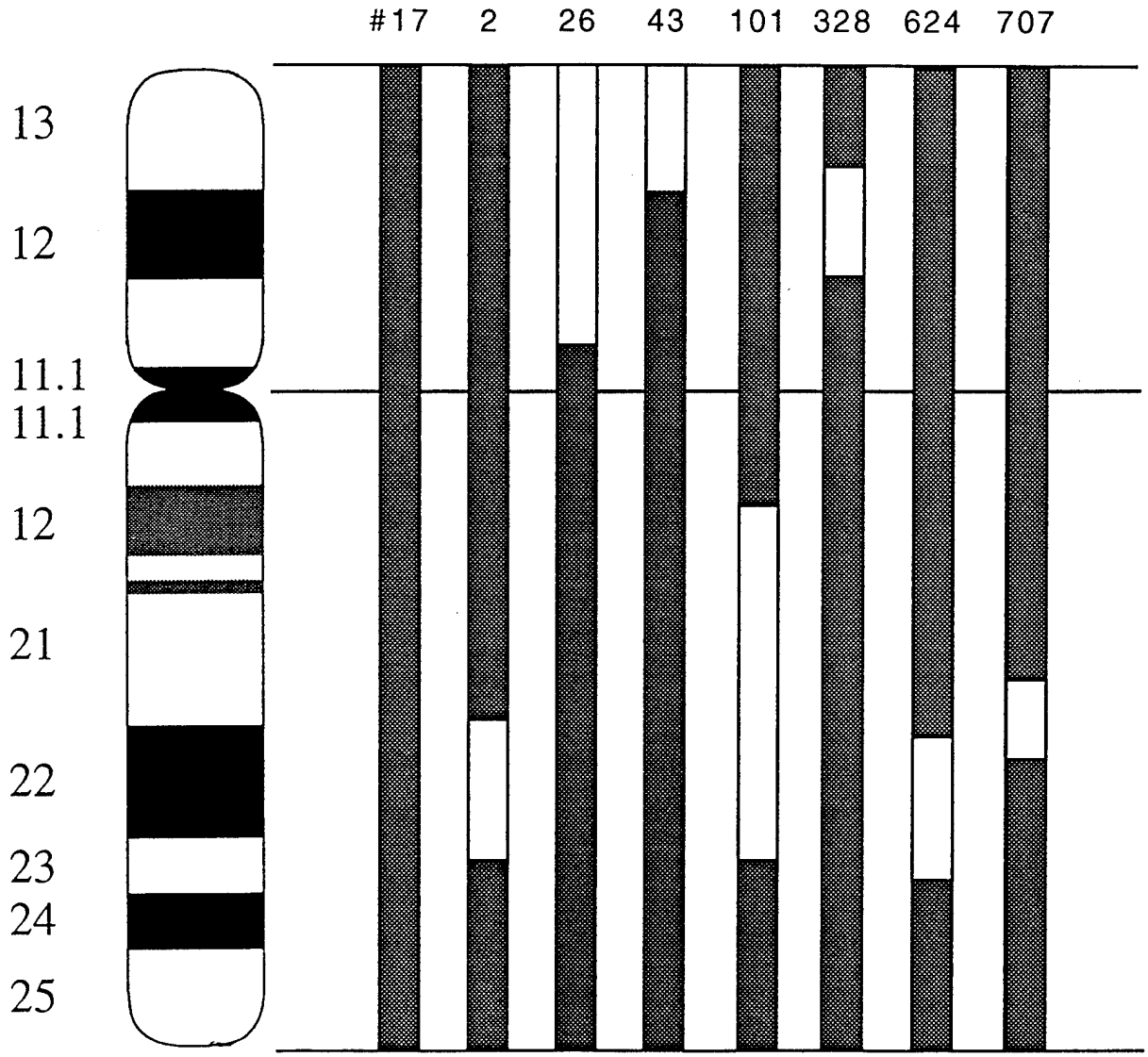
SSTR2

Following microcell transfer of deleted chromosomes, microsatellite analyses are performed to determine retention or loss of the introduced chromosome 17 using up to 20 markers that distinguish the MCF7 chromosome 17 from the donor microcell hybrid chromosome 17. Our hypothesis is that the majority of clones will retain an introduced chromosome or region of chromosome 17 if it does not contain the growth arrest gene.

In this figure, neomycin-resistant MCF7 clones were analyzed for the presence of the SSTR2 marker (17q23) by radiolabeled microsatellite analysis following microcell transfer using hybrid 43 which contains a deletion of 17p13.1-pter. MCF7 cells have two SSTR2 alleles, and the introduced chromosome has a single allele which is intermediate in size between the two MCF7 alleles. 14/19 (73%) of the clones retained the SSTR2 marker, which is located on 17q23. The forward SSTR2 primer was end-labeled using T4 kinase, and a PCR reaction performed using DNA from a series of independently isolated neomycin-resistant MCF7 clones. Reactions were resolved on a 8% polyacrylamide gel, and signal detected using a phosphor imager.

FIGURE 2

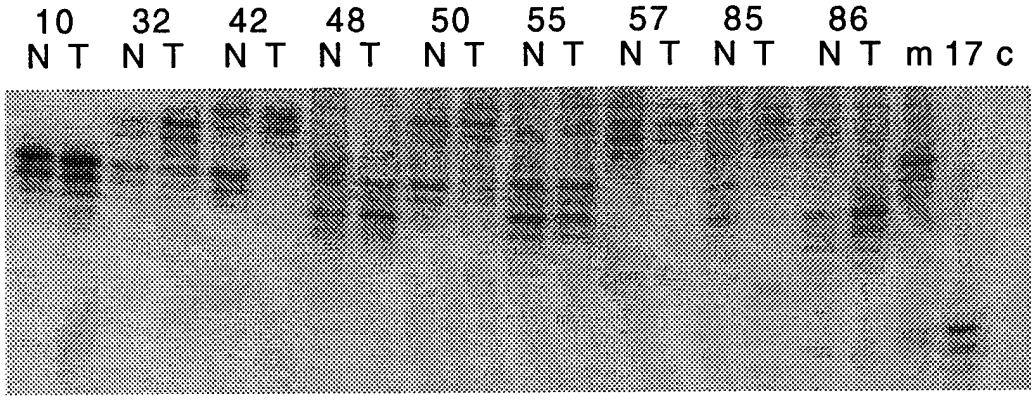
Microcell hybrids currently available for microcell transfer into MCF7 cells



All microcell hybrids shown have been analyzed using a series of over 40 microsatellite markers distributed throughout the chromosome, followed by whole chromosome FISH painting. These chromosomes are currently being introduced into MCF7 cells by microcell transfer, and clones being assayed for growth arrest.

FIGURE 3

Loss of heterozygosity analysis of breast tumor/normal tissue pairs using marker CHRNB1



Loss of heterozygosity analysis of a series of normal/breast tumor tissue pairs using marker CHRNB1 (17p13.1). Loss of heterozygosity is revealed by an absence or reduction in intensity of an informative band in the tumor DNA, and can clearly be seen in samples 42 and 50. The forward CHRNB1 primer was end-labeled using T4 kinase, and a PCR reaction performed using DNA from a series of paired normal/breast tumor samples. Reactions were resolved on a 8% polyacrylamide gel, and signal detected using a phosphorimager. N, normal; T, tumor; m, MCF7; 17, chromosome 17 microcell hybrid; c, control no DNA lane.